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The effect of selected lactic acid bacteria on the microbial composition and on the survival of pathogens in the rumen in context with their probiotic effects on ruminants

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Three year research project

ABSTRACT

This research project was performed in context of the apparent probiotic effect of selected lactic acid bacteria (**LAB**) silage inoculants on the performance of ruminants (improved feed intake, faster live-weight gain, higher milk yields and improved feed efficiency). The overall objective was to find out how LAB affect ruminant performance. The project included several "chapters" as follows:

1. **The effect of LAB silage inoculants on the survival of detrimental bacteria in rumen fluid, *in vitro* study (Weinberg et al.**, The Volcani Center).

An *in vitro* model was developed to study the interaction between selected LAB and an *E. coli* strain tagged with green fluorescence protein (**GFP)** in buffered RF. Results indicated that both LAB inoculants and *E. coli* survived in the RF for several days; both LAB inoculants and LAB-treated silages did not affect survival of *E. coli* in rumen fluid *in vitro*.

2. The effect of feeding baled wheat silages treated with or without three selected LAB silage inoculants on the performance of high-lactating cows (Weinberg et al., The Volcani Center**).**

Treatments included control (no additive), *Lacobacillus buchneri* 40788 (**LB**), *Lactobacillus plantarum* MTD1 40027 (**LP**) and *Pediococcus pentosaceus* 30168 (**PP**), each applied at 10^6 cfu/g FM. The silages were included in the TMR of 32 high milking Holstein cows in a controlled feeding experiment. All baled silages were of good quality. The LB silage had the numerically highest acetic acid and were the most stable upon aerobic exposure. The cows fed the LB silages had the highest daily milk yields, percent milk fat and protein.

3. The microbiome of baled wheat silages and changes during ensiling of wheat and corn (Sela et al., The Volcani Center**).**

Bacterial community of the baled silages was dominated mainly of two genera in total, dominated by *Lactobacillus* and *Clostridium_sensu_stricto_12* with 300 other genera at very low abundance. Fungal community was composed mainly of two genera in total, dominated by *Candida* and *Monascus* with 20 other genera at very low abundance.
In addition, changes in the microbiome during ensiling of wheat and corn with and without addition of *L. plantarum* MTD1 was studied in mini-silos. Overall 236 bacterial genera were identified in the fresh corn but after 3 months *Lactobacillus* outnumbered all other species by acquiring 95% of relative abundance. The wheat silage samples are still under analysis.

4. The effect of applying LAB inoculants at ensiling on survival of *E. coli* O157:H7 in alfalfa and corn silages (**Adesogan et al**., University of Florida).

E. coli (10^5 cfu/g) was applied to fresh alfalfa and corn at ensiling with or without *L. plantarum* or *L. buchneri*. The pathogen was added again after about 3 moths at the beginning of an aerobic exposure period. The inoculants resulted in faster decrease in pH as compared with the control (no additives) or *E. coli* alone and therefore, the pathogen was eliminated faster from these silages. After aerobic exposure the pathogen was not detected in the LAB treated silages, whereas it was still present in the *E. coli* alone samples.

5. **The effect of feeding corn silage treated with or without *L. buchneri* on shedding of *E. coli* O157:H7 by dairy cows** (**Adesogan et al.**, UFL).

Five hundred cows from the dairy herd of the University of Florida were screened for *E. coli* shedding, out of which 14 low and 13 high shedders were selected. These cows were fed a total mixed ration (**TMR**) which was inoculated with *E. coli* O157:H7 for 21 days. The TMR included corn silage treated with or without *L. buchneri.* The inoculated silages were more stable upon aerobic exposure than the control silages; the silage inoculant had no significant effect on any milk or cow blood parameters. However, the silage inoculant tended to reduce shedding of *E. coli* regardless of high or low shedders ($p = 0.06$).

6. The effect of feeding baled wheat silages treated with or without three selected LAB silage inoculants on the rumen microbiome (Mizrahi et al., BGU**).**

Rumen fluid was sampled throughout the feeding experiment in which inoculated wheat silages were included in the rations. Microbial DNA was subsequently purified from each sample and the 16S rRNA was sequenced, thus obtaining an overview of the microbiome and its dynamic changes for each experimental treatment. We observed an increase in OTU richness in the group which received the baled silage inoculated with *Lactobacillus Plantarum* (LP). In contrast the group fed *Lactobacillus buchneri* (LB) inoculated silage resulted in a significant decrease in richness. Lower OTU richness was recently associated in lactating cows with higher performance (Ben Shabat *et al.,* 2016). No significant clustering could be observed between the different inoculation treatments and the control in non metric multi-dimentional scaling, suggesting that the effect of the treatments is not the result of an overall modulation of the microbiome composition but possibly the result of more discrete interactions. Significant phylum level changes in composition also indicates that no broad changes in taxa identity and composition occurred under any treatment

A more discrete modulation could be observed in the fold change of several taxonomic groups (genus level analysis), unique to each treatment, before and after the treatment. Of particular interest is the LB treated group, in which several taxa significantly decreased in abundance.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Reviewed	0	4	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
Ph.D. Student	Ogunade	Ibukun M.	University of Florida	USA
Postdoctoral Fellow	Keshri	Jitendra	The Volcani Center	Israel

Collaboration

The research project comprised 6 distinct chapters which were carried out by the investigators of the project and this resulted in a comprehensive scientific data base pertaining to lactic acid bacteria (LAB) silage inoculants and cow performance. Zwi Weinberg, the PI of the project studied the LAB-E. coli in rumen fluid model system. He also was responsible for baled wheat silages with and without LAB inoculants which were fed to cows and summarized their performance parameters. Adegbola Adesogan from the Univ. of Florida studied the survival of E. coli in silages which were treated with selected LAB inoculants and shedding of E. coli by dairy cows which were fed treated and non-treated silages. Shlomo Sela studied the microbiome of the baled wheat silages and changes of corn and wheat silages (with and without Lactobacillus plantarum) during ensiling. Iztik Mizrahi from BGU studied the microbiome of rumen fluid as affected by feeding the baled wheat silages of the various treatments.

Hence, every investigator in the project contributed his expertise which resulted in a successful project.

It should be mentioned that Zwi Weinberg spent his sabbatical leave with Gbola Adesogan during the project which helped to exchange ideas. The Florida team contributed much to the statistical analysis of the feeding experiment carried out in Israel.

The effect of selected lactic acid bacteria on the microbial composition and on the survival of pathogens in the rumen in context with their probiotic effect on ruminants

Weinberg, Z.G.[1], Sela, S.[1], Mizrahi, I.[2] and Adesogan, A.T.[3]

Final Report of BARD project IS-4704-14 for the activity performed at the [1]Department of Food Science and the Department of Dairy Cattle, The Volcani Center, Israel, [2]Ben-Gurion University, Be'er Sheba, Israel, and [3]Department of Animal Science, University of Florida, Gainesville, Fl., USA.

Achievments

Introduction

This research project was performed in context of the apparent probiotic effect of selected lactic acid bacteria (**LAB**) silage inoculants on the performance of ruminants. Evidence in the literature suggests that silages treated with such inoculants result in improved feed intake, faster live-weight gain, higher milk yields and improved feed efficiency (Kung and Muck, 1997). The overall objective was to find out how LAB affect ruminant performance. For this end the following experiments were performed:

1. **The effect of LAB silage inoculants on the survival of detrimental bacteria in rumen fluid, *in vitro* study (Weinberg et al.**, The Volcani Center).

The objective was to study the interactions between silage LAB inoculants and inoculated silages and of *E. coli* in rumen fluid (**RF**), *in vitro*. Cattle is a natural habitat of *E. coli*, some strains of which are pathogenic to both humans and animals, and therefore, we used *E. coli* as a representative of undesirable bacteria. An *in vitro* model was developed with buffered RF. The RF was added various ruminant feed ingredients inoculated with and without: (a) each of 6 commercial strains of LAB silage inoculants (b) laboratory corn or wheat silages treated with and without these LAB, (c) an *E. coli* strain which has been isolated from cattle manure and tagged with green fluorescence protein (**GFP)**. Results indicated that both LAB inoculants and *E. coli* survived *in vitro* in RF for several days; both LAB inoculants and LAB-treated silages did not affect survival of *E. coli* in rumen fluid *in vitro*. However, when the pH of the RF decreased below

5.0 no viable *E. coli* was detected. This chapter indicates that interactions between LAB inoculants and detrimental bacteria in RF cannot explain the enhancement in animal performance by feeding inoculated silages.

The major results of this chapter were published in Weinberg *et al*., Letters of Applied Microbiology, 2016, 63: 60-65.

2. The effect of feeding baled wheat silages treated with or without three selected LAB silage inoculants on the performance of high-lactating cows (Weinberg et al., The Volcani Center**).**

The main objective of this experiment was to study the effect feeding baled wheat silages inoculated with selected LAB strains on the rumen microbiome. We used this opportunity also to study the effect of the inoculants on the quality of the wheat silages and on performance parameters of high milking cows which were fed these bales. Treatments included control (no additive), *Lacobacillus buchneri* 40788 (**LB**), *Lactobacillus plantarum* MTD1 40027 (**LP**) and *Pediococcus pentosaceus* 30168 (**PP**), each applied at 10^6 cfu/g FM. The silages were included in the TMR of 32 high milking Holstein cows in a controlled feeding experiment. In the initial 10 days all cows received the control silage only, followed by 25 days during which the cows received either the control or one of the treated silages in their rations (8 cows/group). All baled silages were of good quality. The LB silage had the numerically highest acetic acid and the LP the highest lactic acid, as expected. The LB silages were the most stable upon aerobic exposure. The milk yields of the control cows tended to decrease with time due to progress of days in milking (**DIM**), whereas those of the cows which received the silages treated with LB and LP decreased more mildly. The cows fed the LB silages had the highest daily milk yields, percent milk fat and protein. Among the inoculants used, LB resulted in the largest enhancement of lactating cows' performance, followed by LP.

The major results of this chapter were published in Ben-Meir *et al*., Grassland Science (accepted).

3. The microbiome of baled wheat silages and changes during ensiling of wheat and corn (Sela et al., The Volcani Center**).**

The microbiome of the baled wheat silages used in the feeding study was determined using next generation sequencing technology. Bacterial community was dominated mainly of two genera in total, dominated by

Lactobacillus and *Clostridium_sensu_stricto_12* with 300 other genera at very low abundance. In the control samples the genus *Lactobacillus* acquired over 96.6% of total bacterial populations; in LB treated sample *Lactobacillus* were at the abundance of 78.6-99% followed by *Pseudomonas* (2.2%) and unclassified genera (8.35%); in LP treatment, over *97.6%* of sequences belonged to *Lactobacillus* followed by *Weissella* (1.4%); the PP treated sample had 93-96.6% abundance of *Lactobacillus* followed by *Clostridium_ sensu_ strict_12* (1.7%). Fungal community was composed mainly of two genera in total, dominated by *Candida* and *Monascus* with 20 other genera at very low abundance.

In addition, changes in the microbiome during ensiling of wheat and corn with and without addition of *L. plantarum* MTD1 was studied in mini-silos. Overall 236 bacterial genera were identified, *Acinetobacter* (38.5%), *Klebsiella* (16.3%), *Weissella* (14.5%), *Sphingomonas* (10%) were most dominant in fresh corn samples but after 3 months of storage *Lactobacillus* outnumbered all other species by acquiring 94.8% and 97.1% of relative abundance in control and treated samples respectively. After aerobic stability test, *Lactobacillus* slightly increased in both the samples covering 95.5% in control and 99.4% in treated samples. In initial stages of ensiling the significant difference was observed in control and treated samples but after 3 months of incubation, both communities were almost same with dominance *Lactobacillus* (>94%). The wheat silage samples are still under analysis.

A paper has been submitted to Applied Microbiology and Biotechnology.

4. The effect of applying LAB inoculants at ensiling on survival of *E. coli* O157:H7 in alfalfa and corn silages (**Adesogan et al**., University of Florida).

E. coli (10^5 cfu/g) was applied to fresh alfalfa and corn at ensiling with or without *L. plantarum* or *L. buchneri*. The pathogen was added again after about 3 moths at the beginning of an aerobic exposure period. The inoculants resulted in faster decrease in pH as compared with the control (no additives) or *E. coli* alone and therefore, the pathogen was eliminated faster from these silages. After aerobic exposure the pathogen was not detected in the LAB treated silages, whereas it was still present in the *E. coli* alone samples.

Zwi Weinberg from the Volcani Center, Israel, participated in this activity during his sabbatical leave with **Dr. Adesogan** at the Univ. of Florida.

Two papers have been published in the Journal of Dairy Science.

5. **The effect of feeding corn silage treated with or without *L. buchneri* on shedding of *E. coli* O157:H7 by dairy cows** (**Adesogan et al.**, UFL).

Five hundred cows from the dairy herd of the University of Florida were screened for *E. coli* shedding, out of which 14 low (<10^3 CFU/fecal swab)- and 13 high (>10^3 CFU/fecal swab) - shedders were selected. These cows were fed a total mixed ration (**TMR**) which was inoculated with *E. coli* O157:H7 for 21 days. The TMR included corn silage treated with or without *L. buchneri.* The inoculated silages were more stable upon aerobic exposure than the control silages; the silage inoculant had no significant effect on any milk or cow blood parameters. However, the silage inoculant tended to reduce shedding of *E. coli* regardless of high or low shedders ($p = 0.06$). It cannot be concluded that the LAB inoculant effect was more effective with low shedders since the interaction LAB inoculant X shedders was non-significant ($p = 0.69$).

6. **The effect of feeding baled wheat silages treated with or without three selected LAB silage inoculants on the rumen microbiome of high-lactating cows** (**Mizrahi et al.**, BGU).

In order to assess the potential modulation of the rumen microbial community under the different treatments, we sampled rumen fluid throughout the feeding experiment. Microbial DNA was subsequently purified from each sample and the 16S rRNA was sequenced, thus obtaining an overview of the microbiome and its dynamic changes for each experimental treatment.

Microbiome modulation following feeding with baled treated with LAB

16S amplicon sequencing analysis allows for the assessment of the ecological parameters within each feed group. The groups inoculated with both *Lactobacilli* showed a significant change in OTU richness. We observed an increase in OTU richness in the group which received the baled silage inoculated with *Lactobacillus Plantarum* (LP). In contrast the group fed *Lactobacillus buchneri* (LB) inoculated silage resulted in a significant decrease in richness. Lower OTU richness was recently associated in lactating cows with higher performance (Ben Shabat *et al.,* 2016), which was also observed in this experiment with cows receiving the LB treated baled silage exhibiting the highest performance across the different treatments and control. In order to assess whether a broad

modulation of the microbiome can be observed in each treatment we performed a pairwise similarity analysis of the samples using the weighted Unifrac metric, which assess the degree of similarity in composition and phylogenetic similarity of the microbial components in the samples, and separately plotted each treatment group using non metric multi-dimensional scaling (NMDS). In the plot, an observed modulation would result in a significant clustering of samples before and after the treatment with the inoculated feed.

No significant clustering could be observed between the different treatments and the control (figure 2), suggesting that the effect of the treatments is not the result of an overall modulation of the microbiome composition but possibly the result of more discrete interactions. Significant phylum level changes in composition also indicates that no broad changes in taxa identity and composition occurred under any treatment

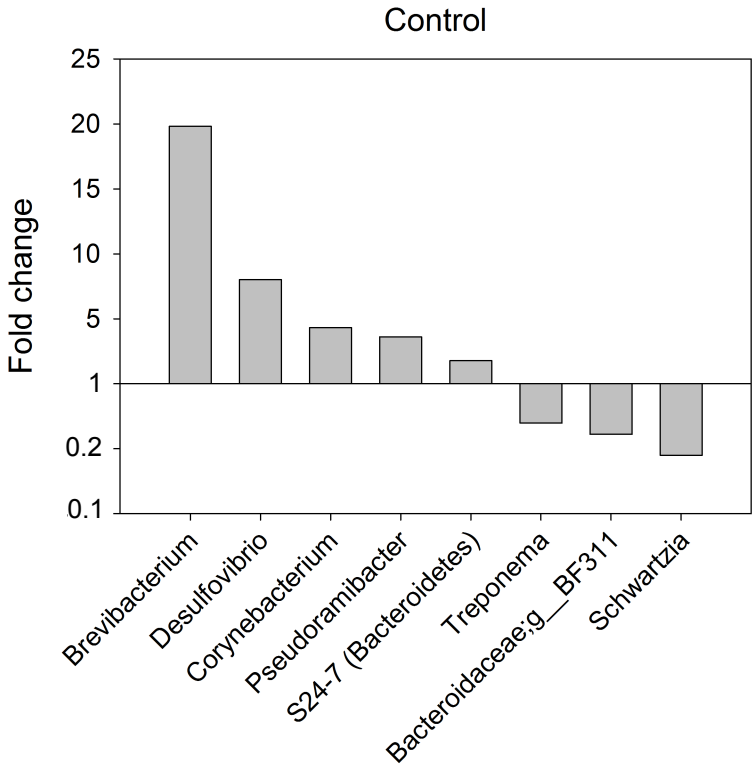
A more discrete modulation can be observed in the fold change of several taxonomic groups (genus level analysis), unique to each treatment, before and after the treatment. Of particular interest is the LB treated group, in which several taxa significantly decreased in abundance.

At this time, the rumen microbiome analysis is still ongoing and the remaining unsequenced samples related to the dynamic changes throughout the experiment are being processed.

Final Conclusions: The project enabled intensive and broad research activity on various aspects of the effect of LAB silage inoculants on animal performance. It produced many interesting results in numerous publications. The rumen microbiome analysis revealed several interesting findings associated with the LB treatment. Coupling these results to the fact that this treatment also resulted the highest cow performance in the current research may lead to the probiotic mode of action of LAB silage inoculants on ruminants. More research is warranted on this topic.

Publications for Project US-4704-14

Status	Type	Authors	Title	Journal	Vol:pg Year	Coun
Published	Reviewed	*Weinberg, Z.G., Chen, Y., Vochinski, V., Sela, S., Ogunade, I.M. and Adesogan, A.T.*	Interactions between LAB silage inoculants and E. coli in rumen fluid in vitro studies	***Journal Applied Microbiology***	:	Joint
Published	Reviewed	*Ogunade, I.M., Kim, D.H., Jiang, Y., Weinberg, Z.G., Jeong, K.C., Adesogan, A.T.*	Control of echerichia coli O157;H7 in contaminated alfalfa silage: effect of silage additives	***Journal of dairy Science***	99 : 4427-4436 2016	Joint
Published	Reviewed	*Ogunade, I.M., Jiang, Y., Kim, D.H., Cervantes, A.P., Ariola, k.G., Vyas, D., Weinberg, Z.G., Jeong, K.C., Adesogan, A.T.*	Fate of Escherichia coli O157:H7 and bacterial diversity in corn silage contaminated with the pathogen and treated with chemical and microbial additives	***Journal of dairy Science***	:	Joint
Accepted	Reviewed	*Ben-Meir, Y., Jamie, E., Chen, Y., Ya'acobi, S., Portnik, Y., Ogunade, I.M., Adesogan, A.T. and Weinberg, Z.G.*	Effect of silage inoculants on the quality of baled wheat silages and cow performance		:	Joint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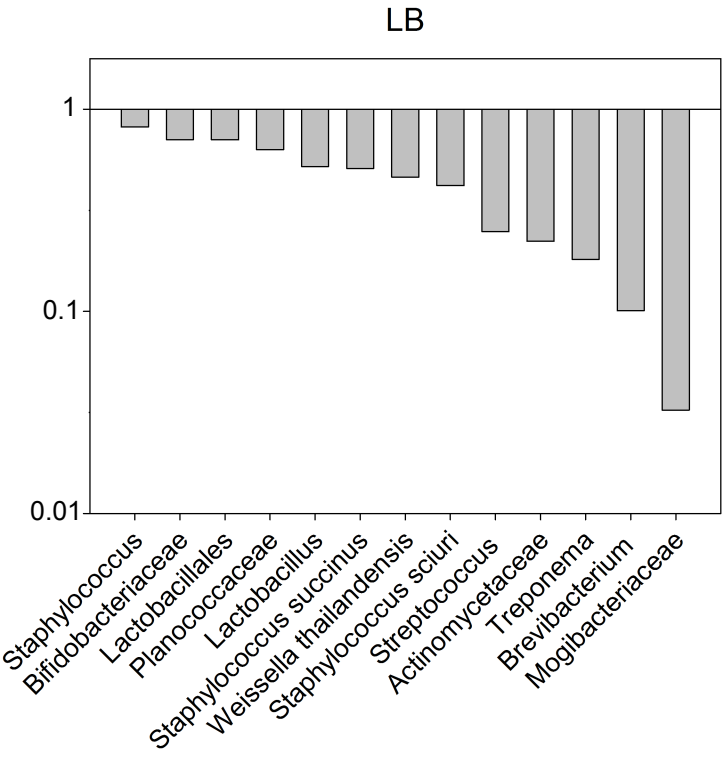
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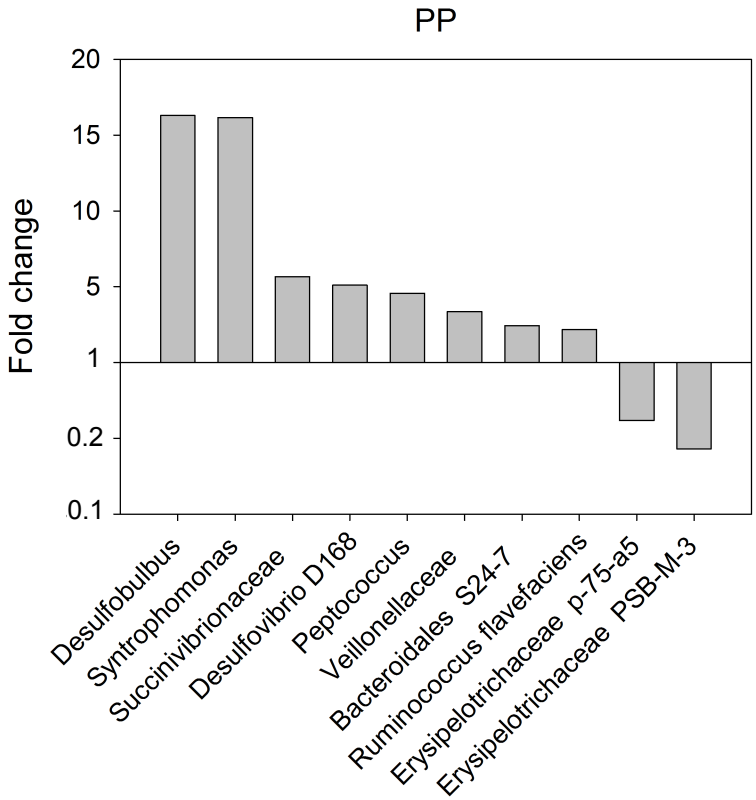
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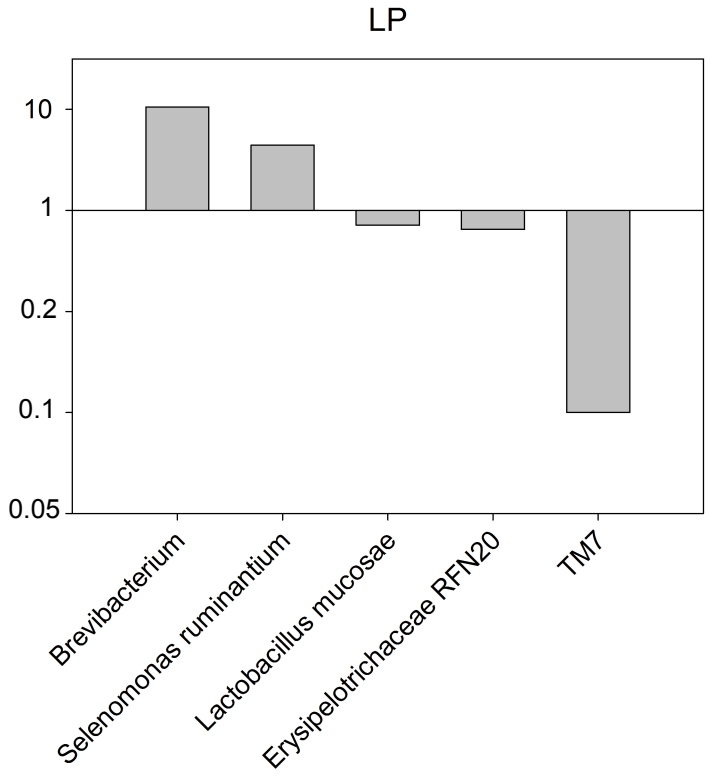
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